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DESORPTION OF ABSORBED IRON IN BEAN ROOT AND LEAF TISSUES

J. H. Jooste and J. A. de Bruyn
(Department of Botany, University of Stellenbosch)

Abstract

The effect of different desorption media on the amount of absorbed Fe (from a solution of $FeCl_3$ in 0,5 mM $CaCl_2$) retained by leaf discs and excised root tips of bean plants was investigated. Attempts were also made to determine the effect of desorption on the intracellular distribution of Fe.

Desorption in water or an $FeCl_3$ solution had no pronounced effect on the amount of absorbed Fe retained by either the leaf or root tissues. However, Na_2-EDTA[1] was able to desorb a considerable portion of the absorbed Fe, especially in root tissue. This applies to Fe absorbed from solutions of $FeCl_3$ and Fe-EDDHA.[2]

Desorption by the chelate removed Fe from practically all the different particulate fractions of both root and leaf tissues, but desorption following the longer absorption periods resulted in an increase in the Fe content of the "soluble" fraction.

The possibility that Na_2-EDTA causes an increased permeability of cell membranes seems likely. The view that removal of Ca by the chelate causes this increase in permeability could not be confirmed.

Uittreksel

DESORPSIE VAN OPGENEEMDE YSTER IN WORTEL- EN BLAARWEEFSEL VAN DIE BOONTJIEPLANT

Die effek van verskillende desorpsiemedia op die hoeveelheid opgeneemde Fe (uit 'n oplossing van $FeCl_3$ in 0,5 mM $CaCl_2$) wat deur blaarskyfies en afgesnyde wortelpunte van die boontjieplant behou word, is ondersoek. Ook is gepoog om die effek van desorpsie op die intrasellulêre verspreiding van Fe te bepaal.

Desorpsie in water of in 'n $FeCl_3$-oplossing het geen noemenswaardige effek gehad op die hoeveelheid opgeneemde Fe wat deur òf blaar- òf wortelweefsel behou is nie. Na_2-EDTA het egter die onttrekking van 'n aansienlike gedeelte van die opgeneemde Fe, veral in wortelweefsel, tot gevolg gehad. Dit geld vir Fe wat uit oplossings van $FeCl_3$ en Fe-EDDHA opgeneem is.

Desorpsie deur die chelaat het Fe uit al die verskillende selfraksies van sowel wortel- as blaarweefsel verwyder, maar desorpsie na langer tydperke van opname het die Fe-inhoud van die „oplosbare" fraksie verhoog.

Die moontlikheid dat Na_2-EDTA 'n verhoogde deurdringbaarheid van selmembrane veroorsaak, lyk waarskynlik. Die opvatting dat verwydering van Ca deur die chelaat hierdie toename in deurdringbaarheid veroorsaak, kon nie bevestig word nie.

Introduction

Jeffreys (1962) concluded that Fe absorbed by discs of potato tissue may be divided into two fractions, viz., EDDHA-removable and EDDHA-resistant. The

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[1] Na_2-Ethylenediamine tetraacetic acid.
[2] Fe-Ethylenediamine di(o-hydroxyphenyl acetic acid).

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former represents intact Fe-EDDHA molecules bound to anion binding sites in the tissues, whereas the latter consists of Fe which has been released by the chelate and bound by the tissues. It is not clear where these two fractions are situated in the cell.

Although cell fractionation methods have been used to study the intracellular distribution of Fe (e.g. by Murphy and Maier, 1967), and desorption treatments have been used to differentiate between active and passive uptake of Fe by enzymically isolated tobacco leaf cells (Kannan, 1969) and by excised rice root tips (Kannan, 1971), no attempts have been made to study the effects of desorption on the intracellular distribution of Fe.

In this investigation different desorption media were employed and attempts were made to determine the effect of desorption on the intracellular distribution of Fe.

MATERIAL AND METHODS

Leaf discs and excised root tips were obtained from bean plants (*Phaseolus vulgaris* L., cv. Top Crop) as previously described (Jooste and De Bruyn, 1979).

Experimental solutions

Unless stated otherwise, experimental solutions consisted of 5 mg l^{-1} Fe (as $FeCl_3$) in 0,5 mM $CaCl_2$ solution, adjusted to pH 3,2 with dilute HCl (Jooste and De Bruyn, 1978), and aerated while maintained at 30 °C. In one investigation, Fe-EDDHA was also used as a source of Fe. With one exception, 2 μCi ^{59}Fe (as $FeCl_3$; Radiochemical Centre, Amersham) were added per litre of experimental solution.

Desorption media

Where desorption treatments were applied, samples were transferred from the experimental solutions to one or more of the following media: water; $FeCl_3$ containing 5 mg l^{-1} Fe, but without ^{59}Fe; and 10 mM Na_2-EDTA. All the desorption media (at least 250 mℓ per sample) also contained 0,5 mM $CaCl_2$ and were maintained at 2 °C. Unless stated otherwise, the desorption period was 30 minutes.

Intracellular distribution of Fe

In these experiments, each sample consisted of 80 leaf discs of 5 mm diameter, or its equivalent fresh mass of excised root tips (c. 0,45 g). After removal from the experimental solution or desorption medium, each sample was rinsed in deionised water for one minute, then immediately placed in liquid nitrogen, and transferred to a freezer (−20 °C) after 1–2 h. The samples were subsequently fractionated according to the method described by Bonner (1965). They were ground in a glass homogeniser, in a medium consisting of 0,30 M sucrose and 0,05 M tris-HCl buffer, pH 7,0, at c. 2 °C, and then subjected to differential centrifugation in an International B-20 centrifuge at 4 °C. Cell walls were precipitated at 25 × g for 5 minutes, nuclei at 100 × g and chloroplasts at 2 000 × g, both for 10 minutes, and mitochondria at 10 000 × g for 20 minutes; the supernatant was regarded as the "soluble" fraction.

For the purpose of radioassay, the amount of ^{59}Fe in the experimental solution was increased from 2 μCi to 20 μCi 1^{-1}.

Following its immersion in an experimental solution or (where used) a desorption medium, each sample which was not to be fractionated, was rinsed in a series of 4 beakers, each containing 200 mℓ deionised water, for a total of one minute. The sample was then dried at 80 °C, wet-ashed, and its ^{59}Fe content determined by means of a well scintillation counter. After making corrections for radioactive decay, the amount of Fe absorbed was calculated.

In determining the Fe content of the different subcellular fractions, the material was not ashed, but transferred to the sintillation vials and made up to 5 mℓ with deionised water. A correction was made for the fact that the material was not equally distributed throughout the medium (Haasbroek, private communication).

Where the Ca content of the tissues was determined, atomic absorption spectroscopy was employed, according to the method of Allen (1970).

Statistics

Two or four replicates of each treatment were used; each experiment was repeated on two consecutive days. Where possible, the mean and standard error for each treatment were calculated. Differences between means of more than twice the standard error were regarded as significant.

RESULTS AND DISCUSSION

In general, desorption in water or an $FeCl_3$ solution had no pronounced effect on the amount of absorbed Fe retained by either the leaf or root tissues (Table 1), after the first hour. During the early stages of absorption, however, a portion of the absorbed Fe appeared to move outwards by diffusion or ion exchange. Na_2-EDTA was able to desorb a considerable portion of the Fe absorbed by the leaves during the first 30 minutes, but not after longer absorption periods; it could, however, desorb the major portion of the Fe absorbed by the roots, even after the longest absorption time of two hours.

In order to determine whether chelated Fe might be more resistant than Fe^{3+} to desorption by Na_2-EDTA, Fe was supplied in the experimental solution at 5 mg 1^{-1} as both $FeCl_3$ and Fe-EDDHA, and the effects of desorption in 10 mM Na_2-EDTA determined. In general, more Fe was absorbed from the $FeCl_3$ solution than from the chelate, but the percentage change in Fe content due to desorption was very much the same (Table 2). Again a smaller percentage of Fe was

TABLE 1.

Effect of different desorption media on the amount of absorbed Fe retained by leaf discs and root tips. Experimental solution contained 5 mg l^{-1} Fe (as $FeCl_3$). Desorption media: water, $FeCl_3$ solution containing 5 mg l^{-1} (without ^{59}Fe), and 10 mM Na_2-EDTA solution. Desorption period 30 minutes at 2 °C. Sample size 0,3 g (fresh mass). Average of 4 replicates.

Absorption period (Min.)	Fe content (μg per sample)							
	Leaf discs				Root tips			
	Desorption treatment				Desorption treatment			
	No desorption	Water	Fe solution	Na_2-EDTA solution	No desorption	Water	Fe solution	Na_2-EDTA solution
10	4,7±0,1	3,1±0,3	3,7±0,2	1,6±0,1	26,7±1,3	22,3±2,4	20,4±0,7	3,7±0,4
30	9,0±0,6	6,3±1,3	7,4±0,1	3,3±0,2	60,8±5,9	43,3±4,1	45,2±8,0	8,4±0,1
60	10,2±0,8	9,8±0,6	9,4±0,0	7,2±1,6	66,1±0,8	54,2±0,9	50,5±2,9	13,9±2,3
120	14,3±0,2	16,0±0,5	17,1±1,5	14,9±0,1	68,1±4,6	69,7±2,0	68,9±5,2	30,0±0,2

desorbed from the leaves than from the roots, especially after the longer absorption periods.

TABLE 2.

Effect of $FeCl_3$ vs. Fe-EDDHA as sources of Fe on the amount of Fe absorbed by leaf discs and root tips, before and after desorption in 10 mM Na_2-EDTA for 30 minutes. Fe supplied at 5 mg l^{-1}; sample size 0,3 g (fresh mass). Average of 4 replicates.

Absorption period (Min.)	Fe content (μg per sample)					
	$FeCl_3$			Fe-EDDHA		
	No desorption	After desorption	% Change	No desorption	After desorption	% Change
	Leaf discs					
10	6,0±0,1	2,7±0,5	−54	3,5±0,8	1,9±0,0	−46
30	12,9±1,1	7,5±0,9	−42	7,9±0,4	4,8±0,8	−39
60	26,5±1,0	16,2±1,6	−39	12,9±0,4	8,7±0,6	−33
120	45,4±6,6	38,7±8,3	−15	22,5±1,0	20,4±2,6	−9
	Root tips					
10	27,8± 2,7	10,7± 0,3	−62	21,1± 5,4	7,2±0,3	−66
30	63,9± 4,0	28,7± 1,7	−55	42,0±10,2	16,2±1,6	−61
60	101,1± 3,1	41,4± 2,1	−59	64,6± 5,9	24,5±1,6	−62
120	138,4±19,2	75,1±14,8	−46	77,9±15,6	41,6±5,0	−46

The possibility that a desorption period of 30 minutes might be insufficient to effect complete desorption of the "removable" Fe was investigated by studying the effects of different desorption periods. Results (not included) indicated that desorption is essentially completed after 5 minutes. Desorption of Fe by Na_2-EDTA therefore appears to be quite rapid.

These findings raised the question: from which part of the cell or tissue is absorbed Fe removed by Na_2-EDTA? The following experiment was therefore carried out: Root tips and leaf discs were allowed to absorb Fe for different periods, after which some of the samples were subjected to a desorption treatment in 10 mM Na_2-EDTA. The plant material was ground up and separated into cell wall, nucleus, chloroplast, mitochondrion and supernatant fractions by means of differential centrifugation. The results of this investigation are presented in Tables 3 and 4.

Table 3 shows that the total amount of Fe per sample as well as the Fe content of practically all the particulate fractions of both leaf and root tissues, decreased as a result of desorption. In contrast, desorption following absorption periods of 60 minutes and longer, resulted in an increase in the Fe content of the soluble fraction (Table 4). More Fe (percentage wise) was withdrawn from the root cell walls and nuclei than from those of the leaf cells (Table 4).

TABLE 3.

Intracellular distribution of absorbed Fe before and after desorption in 10 mM Na_2-EDTA at 2 °C for 30 minutes. Experimental solution contained 5 mg l^{-1} Fe (as $FeCl_3$). Sample size 0,45 g (fresh mass). Average of 2 replicates.

Absorption period (Min.)	Material	Fe content (μg per cell fraction)					
		Without desorption					
		Cell walls	Nuclei	Chloro-plasts	Mitochon.	Soluble	Total
10	Leaf discs	1,8±0,2	1,0±0,1	2,9±0,0	2,0±0,5	2,1±0,5	9,8±0,0
30		4,7±0,8	3,4±0,4	5,5±0,0	3,5±0,8	3,5±0,5	20,6±0,9
60		6,4±1,6	7,0±0,3	10,5±3,2	4,5±0,2	4,1±0,7	32,5±0,7
120		13,5±1,4	9,8±3,6	12,3±0,2	6,4±1,5	5,4±1,2	47,9±0,8
10	Root tips	9,2±2,8	5,1±1,6	—	8,1±1,6	5,5±3,1	27,9±3,5
30		12,9±3,7	9,1±3,1	—	16,2±2,4	7,0±2,1	45,2±5,1
60		15,4±4,2	20,0±2,2	—	26,0±3,2	7,9±1,4	69,3±6,5
120		27,5±9,7	10,2±2,1	—	23,0±4,7	8,1±1,1	68,8±1,7
		After Desorption					
10	Leaf discs	1,4±0,1	0,5±0,1	0,6±0,1	0,3±0,1	1,7±0,1	4,5±0,2
30		2,7±0,3	1,2±0,1	2,0±0,6	0,7±0,0	3,3±0,9	9,9±0,5
60		8,3±0,9	4,4±2,1	4,3±0,2	1,8±0,1	5,6±1,1	24,4±0,3
120		11,5±0,2	4,8±0,6	4,9±0,5	2,3±0,0	7,0±1,0	30,5±1,1
10	Root tips	2,4±0,1	0,6±0,0	—	2,0±0,5	3,9±0,3	8,9±0,7
30		4,2±0,2	1,6±0,9	—	3,1±0,2	7,8±0,6	16,7±1,0
60		6,2±1,3	5,1±1,5	—	6,5±1,2	11,1±1,5	29,0±0,1
120		8,0±0,5	4,7±1,5	—	7,9±0,0	20,3±1,6	40,9±0,7

These results indicate that desorption in Na_2-EDTA removes more Fe, from both root and leaf tissues, than that apparently contained in the apparent free space. This is difficult to explain.

It is generally accepted that Ca is necessary for the maintenance of the differential permeability of cell membranes, and that monovalent ions increase their permeability (Epstein, 1972). According to Foote and Hanson (1964), treatment of soybean root tissue with EDTA causes the removal of two-thirds of the Ca from the tissue. The possibility that a desorption treatment with Na_2-EDTA may remove Ca from the tissue with a consequent damaging effect on the cell membranes, thereby making possible the removal of Fe by the chelate, was accordingly investigated. No difference in Ca content of tissue with and without a desorption treatment, could be detected notwithstanding the presence or absence of Ca in the experimental solution. In addition, no effect of Na^+ ions could be determined. NaCl solutions, with or without Ca, were unable to remove Fe from the tissues—only Na_2-EDTA, with or without Ca in the medium, could do so.

TABLE 4.

Percentage change in the amount of absorbed Fe after desorption in 10 mM Na_2-EDTA at 2 °C for 30 minutes, following absorption periods from 10 to 120 minutes in 5 mg l^{-1} Fe, as $FeCl_3$.

Absorption period (Min.)	% Increase (+) or decrease (−)*										
	Leaf discs						Root tips				
	Cell walls	Nuclei	Chloropl.	Mitochon.	Soluble fraction	Total	Cell walls	Nuclei	Mitochon.	Soluble fraction	Total
10	−24,3	−50,0	−79,2	−84,3	−21,7	−54,8	−74,3	−87,6	−75,7	−28,6	−68,2
30	−41,5	−65,7	−62,8	−81,1	−4,0	−51,6	−67,7	−82,0	−81,1	+11,2	−63,2
60	+29,6	−37,4	−58,5	−60,3	+36,7	−24,9	−59,8	−74,6	−74,9	+42,9	−58,1
120	−14,9	−51,7	−61,5	−63,3	+29,9	−36,2	−70,7	−53,8	−65,8	+151,1	−40,5

*Based on results in Table 2.

Removal of Fe which has moved through differentially permeable membranes, apparently occurs during desorption in Na_2-EDTA. This was indicated by the finding that withdrawal of Fe from cell organelles like nuclei, chloroplasts and mitochondria took place during desorption. The possibility that cell membranes become more permeable as a result of desorption seems likely. The view that removal of Ca by the chelate causes this increase in permeability could not be confirmed. It would also appear that Na^+ ions play no role in the seemingly increased permeability of the cell membranes.

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